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Physics and the canalization of morphogenesis: a grand challenge in organismal biology

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Abstract

Morphogenesis takes place in a background of organism-to-organism and environmental variation. Therefore, a fundamental question in the study of morphogenesis is how the mechanical processes of tissue movement and deformation are affected by that variability, and in turn, how the mechanics of the system modulates phenotypic variation. We highlight a few key factors, including environmental temperature, embryo size, and environmental chemistry that might perturb the mechanics of morphogenesis in natural populations. Then we discuss several ways in which mechanics – including feedback from mechanical cues – might influence intra-specific variation in morphogenesis. To understand morphogenesis it will be necessary to consider whole-organism, environment, and evolutionary scales because these larger scales present the challenges that developmental mechanisms have evolved to cope with. Studying the variation organisms express and the variation organisms experience will aid in deciphering the causes of birth defects.

Keywords

Developmental biomechanics; morphogenesis; cellular mechanics; phenotypic variation; phenotypic plasticity; eco-devo; birth defects; evo-devo; robustness

Introduction

In nature, all organisms – whether human, invertebrate, or plant – exist in the presence of genetic and environmental variation, as well as developmental noise. Therefore, whether our interest is the causes of human birth defects or the larval ecology of mudflat snails, we face the same problems: how do developmental mechanisms cope, or fail to cope, with the variation the organism experiences? And what determines the distribution of morphologies organisms exhibit in natural populations? Numerous studies have investigated factors likely to be significant to these questions in the context of molecular regulatory pathways (e.g. (Rutherford and Lindquist, 1998, Gilbert, 2001, Houchmandzadeh et al., 2002, Nijhout, 2003, Gilbert, 2005, Pei et al., 2007, Yeyati et al., 2007, Braendle and Felix, 2008, Layalle et al., 2008)), but few studies have investigated how the mechanics of morphogenesis affects phenotypic variability and how the mechanics of morphogenesis is itself affected by environmental variability.

Morphogenesis is a mechanical process since it is the movement and deformation of a material structure (Koehl, 1990). In any material, the applied forces, and the resistance to those forces determines the rate and magnitude of deformation (Box 1). Both the forces and resistances acting within a structure depend on the geometry of the structure and the spatial

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distribution of the applied forces. Therefore, any change in the rate or geometry of morphogenesis must involve a change in the mechanics of the system.

We argue that an integrative approach, with a focus on the whole organism in its environment, will produce insight into the mechanics of morphogenesis and the causes of developmental defects. To illustrate this, we focus on two related strands. First we discuss a few of the sources of variation that might interact with the physical process of morphogenesis. Then we ask how the mechanics of morphogenesis could impact variation in the resulting morphology or sensitivity to perturbations. In addition, we discuss a few cases in which development is remarkably robust to variation in initial conditions.

Much work has been done to investigate how gene-regulatory networks remain robust to perturbations of various kinds, providing insight into fundamental principles governing such networks (e.g. (von Dassow et al., 2000, Nijhout, 2002, Ma et al., 2006, Veitia and Nijhout, 2006, Braendle and Felix, 2008)). Considerations of the real-world variation that organisms experience is likely to provide important insights in developmental biomechanics while integrating it more effectively with the study of birth defects on the one hand, and the evolution and ecology of development on the other. Our principle focus here is on interactions between mechanics and development in animals, because this is the area in which we are most familiar; however, we expect insight could be gained from even broader comparisons.

Sources of mechanical variation

One of the few universal facts in biology is that all organisms exhibit variation. The importance of this has been recognized at least since Darwin's Origin of Species. Since then, the fields of evolutionary biology, ecology, population genetics, and epidemiology have extensively investigated the sources of natural biological variation. Some of the major sources of variation include genetic variation, phenotypic plasticity (environmental), developmental noise, as well as ontogeny and aging. The last of these -- aging -- would not be directly significant for the embryo, but could be indirectly important because maternal condition influences egg condition. We still know little about either the magnitude or the sources of natural variation in embryo mechanics, but a few studies have attempted to rigorously quantify embryo-to-embryo or clutch-to-clutch variation in the mechanical properties of embryos or embryonic tissues. These studies indicate substantial variation in tissue compliance in gastrula stage frog and sea urchin embryos (von Dassow and Davidson, 2007, von Dassow and Davidson, 2009) and in the apparent surface tension in frog cell aggregates (Kalantarian et al., 2009).

The interactions between sources of variation can provide new insights into developmental mechanisms. For example studies of changes in the effect of different mutations at different temperatures or nutritional states provide insight into the role of different genes and gene pathways (Pei et al., 2007, Braendle and Felix, 2008). Also, numerous studies have investigated the existence and nature of the “phylotypic stage,” a stage during development at which different members of a large taxonomic group tend to look more similar to each other than they do at earlier or later stages. Several cross-species studies suggest that the original concept of a phylotypic stage is not well supported (Richardson, 1995, Bininda-Emonds et al., 2003, Poe and Wake, 2004). However, intra-specific studies looking at intrinsic and environmentally-driven variation have also been done to investigate whether this period - or any other - represents a particularly sensitive period in development, or alternatively, a particularly robust period in development (Galis and Metz, 2001, Irmeler et al., 2004, Schmidt and Starck, 2010). These intra-specific studies reveal aspects of the large-scale organization of development, such as ontogenetic changes in the degree of

interdependence, connectivity and modularization among parts of the organism, which are not revealed without considering phenotypic variation.

A few of the major environmental sources of variation that could influence the mechanics of morphogenesis are variation in developmental temperature, egg or embryo size, and chemical environment. These factors are important in evolutionary and ecological contexts since most natural populations encounter variation in each of these. Variation in developmental temperature and chemical environment are known to be important in clinical settings as well. A vast literature – which we shall not review here – discusses the ecological impacts of each these factors.

Temperature and mechanics

Most animals are ectothermic, and ectotherms typically exhibit strong dependence of developmental rate on temperature (Bachmann, 1969, Gillooly et al., 2002, Dixon et al., 2009). In most cases there is a range of temperatures over which most embryos develop normally, but faster at higher temperatures and slower at lower temperatures. However, there are sharp upper and lower thresholds beyond which development fails (Bachmann, 1969, van der Have, 2002, Dixon et al., 2009). Most natural populations are exposed to a wide range of different temperatures across their range, and individual embryos can experience quite rapid, large ($>10^{\circ}\text{C}$) temperature changes in natural environments (Podolsky 2003, Kaplan and Phillips, 2006). The change in developmental rate across temperatures is dramatic: *Xenopus laevis* develops about three times faster at 28°C than at 16°C (Khokha et al., 2002). Based on our assumption (above) that to change the rate of material deformation requires a change in either the forces driving or the forces resisting the deformation, we predict that the temperature dependent change in morphogenetic rate will involve changes in tissue viscoelasticity or force generation.

Endotherms, including humans, are not completely free from variation in body temperature. Variation in temperature is associated with developmental defects in several species of mammals (reviewed in (Edwards, 2006)), and epidemiological studies suggest that exposure to high body temperature (e.g. from fever, or exposure to unusual high external temperature) is a major risk factor for a wide variety of human birth defects (Milunsky et al., 1992, Chambers et al., 1998, Acs et al., 2005, Moretti et al., 2005, Edwards, 2006). The effect of fever appears to be due to the temperature, rather than infection (Acs et al., 2005). Several hypotheses for why temperature causes developmental defects have been proposed (Nelson et al., 1982, van der Have, 2002, Edwards, 2006, Hosako et al., 2009), but to the best of our knowledge, the contribution of mechanics has not been considered.

Normal development involves a large number of interactions among different tissues occurring at specific times. Therefore, we expect that normal morphogenesis requires coordinated changes in the rates of different developmental movements and the timing of patterning events occurring in different parts of the embryo. Given that morphogenesis involves a balance of force generation and resistance to deformation, we further expect that tight coupling between temperature driven changes in force generation and temperature driven changes in the resistance to deformation would be required to maintain the coordination of these processes. Developmental defects might occur when this coupling breaks down.

Many studies indicate that temperature has pronounced effects on cell viscoelasticity and compliance (Norris, 1940, Mitchison and Swann, 1954a, Sung et al., 1982, Evans and Yeung, 1989, Liu et al., 2007, Picard and Donald, 2009, Sunyer et al., 2009b, Rico et al., 2010), traction force generation (Sunyer et al., 2009a, Sunyer et al., 2009b) or cortical tension (Liu et al., 2007), and cell adhesion (Rico et al., 2010). Although most of these

studies have been done with cultured human cells, and most have been done over temperature ranges beyond those one would expect human embryos to experience, some studies have been done in ectothermic organisms as well (Norris, 1940, Mitchison and Swann, 1954a), as well as numerous studies of the effect of temperature on muscle in ectotherms (reviewed in (Bennett, 1984, Rall and Woledge, 1990)). The magnitude and direction of change viscoelasticity and force is not always consistent, perhaps due to differences in cell types, temperature ranges, and methodology, but the effects can be quite substantial. For example, Sunyer et al. (Sunyer et al., 2009b) found that alveolar epithelial cells were substantially stiffer and less fluid like, and exerted twice as much traction force at 37°C than at 13°C. Liu et al (Liu et al., 2007) found a four fold increase in cortical tension and two fold decrease in viscosity from 22°C to 37°C in neutrophils. And Rico et al (Rico et al., 2010) found a fourteen fold *decrease* in Young's modulus and an eight fold increase in the work of de-adhesion from 16°C to 37°C in monocytes. These three factors – viscoelasticity, force generation, and cell adhesion – are central to driving morphogenesis, so we might expect that temperature driven changes in each of them would have profound effects on development.

Our expectation (above) that force generation and mechanical resistance must be coordinated to maintain normal development as temperature changes is based on the assumption that the relative timing of developmental events remains constant as developmental rate changes. Several studies support this (Bachmann, 1969, Jarosik et al., 2002, Jarosik et al., 2004). However, studies on developmental rate typically focus on time scales comprising large developmental periods. Multiple morphogenetic processes can occur within a single developmental stage, and there can be large individual-to-individual variation in the relative rates of these processes (Ewald et al., 2004). One study indicates that the relative rates of nuclear movement and nuclear division vary with temperature in wasp embryos (Niemuth and Wolf, 1995). One way development could tolerate variation in the relative rates of different morphogenetic processes would be to have broad windows of time and broad morphogenetic fields within which inductive events can occur. As long as each morphogenetic movement gets the tissue *near* the normal position, the next developmental events could proceed. So, while the average developmental rate may change smoothly with temperature, it remains possible that individual morphogenetic processes are not as coordinated within each stage: there may be enough buffering against variation in timing to compensate for changes in the relative rates of different movements. In this case morphogenetic defects might occur when the differences in relative rates among different kinds of developmental processes becomes too large to compensate for. In either case, we expect that cell and tissue mechanics would play a major role in determining the temperature dependence of both developmental rate and developmental success.

Placing embryos in temperature gradients provides a powerful approach to studying the sensitivity of development to variations in synchrony among parts of the embryo and the mechanisms that coordinate development (Niemuth and Wolf, 1995). Temperature gradients were first used to test whether gradients in metabolism drove patterning in the embryo (Huxley, 1927, Dean et al., 1928, Gilchrist, 1928, Tazelaar et al., 1930, Black, 1989), and have subsequently been used to investigate the coordination of bicoid gradients, gap gene, and pair-rule gene expression in *Drosophila* (Lucchetta et al., 2005, Lucchetta et al., 2008) and somitogenesis in zebrafish (Jiang et al., 2000).

Remarkably several studies indicate that changing the relative rates of development in different parts of the embryo can result in surprisingly slight morphological defects (Black, 1989, Niemuth and Wolf, 1995, Black et al., 1996). In embryos of the frog *Xenopus laevis*, the blastopore first appears as bottle cells contract on the dorsal side of the embryo, subsequently bottle cell contraction occurs on the lateral sides, and finally on the ventral

side. A gradient in temperature applied during cleavage stages, sufficient to reverse the normal dorsal-to-ventral progression of blastopore formation, resulted in fairly normal neurula stage embryos and tadpoles (Black, 1989). Similar results were obtained using localized hypoxia to produce a gradient in developmental rate (Black et al., 1996). One remarkable study shows very similar results with wasp embryos (Niemuth and Wolf, 1995). Temperature gradients were used to produce a four-fold change in developmental rate between the anterior and posterior of the embryo for long periods during cleavage, cellularization, or gastrula stages, yet the embryos were able to still develop and hatch. Niemuth and Wolf (Niemuth and Wolf, 1995) found that the embryos were able to regulate their development so that parts of the embryo that were experimentally slowed down could catch back up to the faster parts.

Given the great interest in robustness, developmental modularity and heterochrony, particularly in the study of the evolution of development (Richardson, 1995, von Dassow and Munro, 1999, Bolker, 2000, Chipman et al., 2000, Galis and Metz, 2001, Bininda-Emonds et al., 2003, Gibson and Gibson, 2004, Poe and Wake, 2004, del Pino et al., 2007, Shook and Keller, 2008, Schmidt and Starck, 2010), it is unfortunate that the fascinating studies on driving gradients in developmental rates within embryos have gone largely unnoticed. While all of these studies take great pains to emphasize that these gradients are highly unnatural conditions, we wonder whether a large egg deposited on a sun warmed surface in air might experience some small degree of a temperature gradient; or perhaps oxygen gradients found in egg masses (e.g. of amphibians, fish, or invertebrates (Cohen and Strathmann, 1996)) could get steep enough to produce some degree of developmental desynchronization within large embryos. Certainly these studies make us question our assumption that embryos require tight regulation of mechanical behavior to produce successful development, and they bear directly on the hypothesis that mechanical interactions among parts of the embryo induce and coordinate morphogenesis (Farge, 2003, Supatto et al., 2005, Beloussov et al., 2006, Desprat et al., 2008, Taber, 2009). They leave us with the question: given that mechanical processes are universally dependent on boundary conditions and geometry, how can morphogenesis tolerate great disruptions in its geometry (Harris, 1987)?

Egg or embryo size and other maternal effects

Egg or embryo size is also variable within populations (Kaplan and Phillips, 2006, Marshall and Bolton, 2007); Fig. 1), and this size variation could modify the physics of morphogenesis in interesting ways. Mammal embryos also exhibit substantial size variation at early developmental stages (Rands, 1986, Richter et al., 2001). Since egg size depends non-linearly on maternal temperature in a number of systems (Bownds et al., 2010), these two factors could combine in complex ways.

Several models assume that surface tension-like forces acting on a fluid-like embryonic material drive morphogenetic movements. These models include the differential adhesion hypothesis (Steinberg, 1963, Foty et al., 1994, Foty et al., 1996, Davis et al., 1997) and the differential contractility hypothesis (Harris, 1976, Krieg et al., 2008), as well as variants on those (Harris, 1976, Manning et al., 2010). These models predict that morphogenetic rates should slow down with increasing embryo or tissue size (Gordon et al., 1972, Mombach et al., 2005, Grima and Schnell, 2007). Simple dimensional analysis indicates that if we have a model including only a surface tension, T , a viscosity, μ , and a tissue radius, r , then the time, t , to complete a change in shape would be proportional to the radius:

$$t \propto r \cdot \mu / T \quad (1)$$

Hence these models predict that morphogenetic movements should proceed more slowly for large embryos than for small embryos, assuming the viscosity and surface tension remain constant. Unfortunately, while several studies have looked at variation in the timing of development with egg or embryo size (Mita, 1983, Rands, 1986, Power and Tam, 1993, Staver and Strathmann, 2002, Marshall and Bolton, 2007), we are not aware of within-species studies focusing on a small and specific enough developmental periods to make conclusions about the dependence of the speed of specific morphogenetic movements on embryo size.

Movements driven by other mechanisms would also depend on embryo or tissue size. For example, the mechanics of notochord straightening is related to the physics of beams (Adams et al., 1990, Koehl et al., 2000). While this work focused on the effect of notochord structure, the various possible modes of deformation, including Euler buckling and kinking, depend on different powers of beam length and diameter, as does the tension in the notochord sheath for a given pressure. Therefore the capacity of the notochord to deform its surroundings will depend on the size of the notochord.

A wide variety of other mechanical processes will similarly depend on the size of the structure. One case includes the stress on structures that act as pressure vessels. Examples relevant to morphogenesis include the expanding vertebrate brain (Alonso et al., 1998, Desmond et al., 2005), the notochord (as described above; (Adams et al., 1990)) and sea urchin embryos during gastrulation (Takata and Kominami, 2001b). In each of these cases, successful development appears to be related to maintaining the correct amount of internal pressure. If the thickness of a vessel wall and the internal pressure do not change with vessel size, then the stress on the wall will increase in proportion to the vessel size. Constancy of tissue thickness would be expected if cell size and the number of cell layers are regulated separately from total embryo size.

Naturally, maternal effects of a wide variety of other kinds could also contribute to mechanical variation in the egg. Many of the mechanically important cytoskeletal proteins, such as actin and myosin, which are necessary for early cleavage divisions, are present in the egg (Valentine et al., 2005, Bendix et al., 2008). We, and others, have observed substantial variation in the mechanical properties of embryos at blastula and gastrula stages, much of which appears to be real embryo-to-embryo or clutch-to-clutch differences, not simply noise (von Dassow and Davidson, 2007, Kalantarian et al., 2009, von Dassow and Davidson, 2009). Some of this variation could result from variation in the maternal contribution of cytoskeletal proteins. However, further studies are sorely needed to confirm or refute these results, and the relative contributions of maternal and zygotic components to this variation remain to be determined.

Chemical environment

Finally, variation in the chemical environment could be a significant factor in altering the mechanics of morphogenesis. In many marine organisms, the salinity of the environment could alter the osmotic pressure within the embryo, thereby altering the forces on the tissues. In some echinoderms osmotic pressure has been shown to affect the progress of gastrulation (Moore, 1941, Takata and Kominami, 2001b), though it's not clear if the embryos would normally encounter this effect in nature. Related to this is the variable formation of wrinkled versus unwrinkled blastulae in some species of echinoderms: some batches of embryos express this phenotype in natural seawater, but the frequency increases as salinity decreases (Kobayakawa and Satoh, 1978). We expect the difference between wrinkled and smooth blastulae to reflect differences in the forces in the blastula wall prior to gastrulation, although the resulting gastrulae were smooth in both wrinkled and unwrinkled blastulae.

Naturally, a wide variety of other environmental chemicals could influence the mechanics of morphogenesis. For example, ion concentrations vary greatly among different bodies of fresh water, and variation in "egg quality" in *Xenopus* is associated with the concentration of calcium and magnesium in the water in which the mother is reared (Godfrey and Sanders, 2004). Since calcium is important in cell adhesion and cell contractility, among numerous other processes, variation in calcium could influence embryo mechanics.

It is unknown whether biomechanically-mediated effects of environmental conditions contribute to developmental defects in natural populations, including humans. Even still, it is clear that a wide variety of environmental factors – both chemical and physical – are likely to influence the mechanics of morphogenesis, as will the intrinsic variation of the embryo due to genetic and maternal effects. Furthermore, these changes in mechanics could contribute to phenotypic variation. We expect that understanding how these factors influence developmental biomechanics will help both to elucidate why the mechanisms of morphogenesis are what they are, and to identify potential contributing factors for developmental defects in animals and humans.

Mechanics of morphogenesis and variation

Now that we have considered a few of the many possible sources of mechanical variability which could be encountered by natural populations, we should consider how the mechanisms of morphogenesis might translate variation in mechanical properties, developmental timing, and structure into variation in morphology. We focus on five features that might make the physical process of morphogenesis more robust to perturbations. (1) Coupling force generation to the resistance to deformation could allow changes in force generation to be balanced by changes in the resistance to deformation. (2) Different geometries for the mechanisms driving morphogenesis can produce different degrees of sensitivity to changes in force generation or viscoelastic resistance. (3) The specific form of viscoelastic resistance could also affect the degree of robustness. (4) Feedback mechanisms could operate to tune the developing structure. (5) Finally, several mechanisms have been identified (e.g. differential adhesion and differential contractility) that could produce the same final structure starting from a wide range of initial configurations. In the following sections we address how these features may affect the robustness of morphogenesis.

(1) Coupling force to the resistance to deformation

Previous studies suggest that embryonic tissues exhibit substantial mechanical variability. However, in many cases the same molecular pathways modulate both force generation and the resistance to deformation (reviewed in (Davidson et al., 2009)). The most common example is the actin-myosin cytoskeleton. Ideally, increased F-actin polymerization and myosin activity in the embryo could increase force generation and the resistance to deformation coordinately, so that the deformations of morphogenesis would be minimally perturbed by moderate changes in the F-actin and myosin states. Preliminary studies in *Xenopus* embryos suggest that force generation and the resistance to deformation are positively correlated, and that this coupling may be based on actomyosin regulation (Rolo et al., 2009, Zhou et al., 2009, von Dassow et al., 2010, Zhou et al., 2010b, Zhou and Davidson, (submitted)). Actomyosin is a major controller of both tissue resistance to deformation and force generation in these embryos, so this mechanism may buffer embryos from perturbations in cell mechanics.

Such coupling between force generation and resistance to deformation is known to occur in some cultured cell types (Wang et al., 2002, Engler et al., 2006, Sunyer et al., 2009b) but may not occur in others (Poh et al., 2010). In addition, the concentration and expression of F-actin cross-linkers appears to be crucial to generating the link between myosin activity,

force generation, and resistance to deformation (Bendix et al., 2008, Kasza et al., 2009, Koenderink et al., 2009). Together these observations suggest that genes which determine the expression of F-actin cross-linkers could modulate the coupling between force and resistance to deformation, and therefore could affect the sensitivity of morphogenesis to variation in cell mechanics. If mutations in these genes alter the strength of coupling between force generation and resistance to deformation, they should change the sensitivity of morphogenesis to other perturbations in cell mechanics. In addition, we expect that genotypes which have higher sensitivity to perturbations in cell mechanics (e.g. due to variation in environmental conditions) will exhibit higher frequencies of morphogenetic defects, and therefore lower fitness. Therefore, we hypothesize that natural selection could act upon the expression levels of F-actin cross-linkers to reduce the sensitivity of morphogenesis to variation in cell mechanics.

This is not the only example of coupling between force generation and resistance to deformation. In later developmental stages, the *Xenopus* notochord extends as it becomes pressurized. The increasing pressure also increases notochord stiffness, so notochord stiffness and force generation go up together (Adams et al., 1990, Koehl et al., 2000). However, in this instance, the coupling would not necessarily stabilize morphogenesis since the compliance of the surrounding tissue may not be coupled to force generation by the notochord.

(2) Geometry and robustness

Several studies suggest that the structure of the embryo, and the geometrical arrangement of the forces acting on it, can affect the sensitivity of morphogenesis to variation in the magnitude of the forces and the tissue properties. For example, in sea urchins the initial invagination of the archenteron could theoretically be driven by several distinct mechanisms (Davidson et al., 1995). In one of these, swelling of the innermost layer of the extracellular matrix (ECM) that lies outside the cellular layer of the embryo would bend the ECM inwards, pushing the cells ahead of it. In another, contraction of the cell apices could cause the cells to become wedge-shaped, thereby bending the cell layer inwards. Each of these mechanisms could drive primary invagination over broad ranges in the ratio of ECM- to cell-stiffness, and within these ranges the predicted morphology is often fairly insensitive to the ratio of ECM- to cell-stiffness. However, these ranges differ considerably among mechanisms, and can be mutually exclusive, due to differences in the geometries of the forces acting on the structure of the embryo. Some models of fruit fly ventral furrow invagination and chick head fold formation suggest that these processes could also occur with little variation over broad ranges of mechanical parameters (Conte et al., 2009, Varner et al., 2010), but neurulation may be less robust to mechanical variation (Chen and Brodland, 2008).

Providing redundancy by combining complementary mechanisms that drive similar movements may also make morphogenesis more robust to perturbations. In both dorsal closure in fruit flies (Kiehart et al., 2000, Hutson et al., 2003, Toyama et al., 2008), and blastopore closure and endoderm internalization in *Xenopus* (Keller, 1984, Winklbauer and Schurfeld, 1999, Ewald et al., 2004, Keller and Shook, 2004) there is evidence that multiple cellular processes acting in different parts of the embryo contribute to driving the morphogenetic movement. Interference with one or more of these mechanisms slows down or alters, but does not necessarily prevent, the morphogenetic movement.

As a simple example of how the geometry of a system could contribute to the robustness of morphogenesis, one way to obtain a shape change in an elastic system that is largely insensitive to variation in the forces would be for the system to exhibit snap-through buckling as illustrated by an analogy with a pair of hinged elastic bars (Figure 2). Here

loading the bars will cause a small deflection as the bars get compressed, up to a critical point at which further deflection allows extension of the bars. At that point the system snaps to a new stable configuration. In this system, one would get fairly similar degrees of deflection with a broad range of force/stiffness ratios, as long as the force/stiffness ratio was above the critical load.

(3) The form of viscoelasticity

Materials can show a wide range of mechanical properties, and these properties could influence how variation in tissue mechanics, force generation and timing of developmental events leads to variation in morphogenesis. Consider for example how morphogenesis might be influenced by variation in the timing of developmental events. Many developmental events involve signaling events that occur when one tissue (A) moves within range of responding tissue (B). These signaling events have to occur in the right time period for normal development, so tissue A has to move or deform by an amount sufficient to reach tissue B when tissue B is competent to receive the signal. A classical example is the "splitting" of the vertebrate eye field by the anterior-ward movement of medial tissues. These tissues carry with them a source of sonic hedgehog which inhibits "eye-expression" at the midline and causes the generation of two separate eyes rather than a single large cyclopic eye (Chiang et al., 1996). If the medial tissues do not extend to the right point at the right time, a cyclopic eye forms instead of the normal two eyes (Varga et al., 1999). Our question then is: how does the viscoelastic behavior of the tissue affect the sensitivity of the embryo to variation in timing of the start of the movement of tissue A, or in the timing of the competent window of tissue B?

To begin with we can simplify this to a 1D, linear, small deformation case. We assume that movement of tissue A is driven by a force (or stress, σ) applied to the system as a step function at time t_0 . We also assume that morphogenesis is quantifiable as a strain (ϵ) at time t_1 . Therefore, we consider the variation in ϵ as a function of the time delay, t , between the force application and a second developmental event, the competent window of tissue B. If the tissue acts as a Newtonian fluid with viscosity μ , then the strain is as follows:

$$\epsilon(t) = t \cdot \sigma / \mu \quad (\text{eqn. 2})$$

On the other hand, if the tissue behaves as a purely elastic material, with Young's modulus E :

$$\epsilon(t) = \sigma / E \quad (\text{eqn. 3})$$

Obviously for the viscous case, the resulting morphology strongly depends on time, but for the elastic case it does not depend on time. Returning to the temperature dependence of developmental rate (discussed above) if the tissue behaves as an elastic solid, the ratio of stress to Young's modulus should remain constant with changes in temperature in order to get the observed changes in developmental rate; but if the tissue behaves as a viscous liquid, the ratio of stress to viscosity should change in direct proportion to the change in developmental rate.

To illustrate the effect of viscoelasticity, we consider the particular case of power-law viscoelasticity, because it spans a range from purely elastic to purely viscous behavior, and has been observed in cells and embryos. Other viscoelastic models could be analyzed in a similar way. For the power-law model we have:

$$\varepsilon(t) = J_0 \cdot \left(\frac{t}{\tau}\right)^\beta \cdot \sigma \quad (\text{eqn. 4})$$

For small variances in timing we can take the ratio, S , of the perturbation in ε to the perturbation in t , scaled to the magnitude of ε and t as a crude measure of the sensitivity of the system to variation in timing:

$$S = \frac{d\varepsilon}{dt} \cdot \frac{t}{\varepsilon} = \beta \quad (\text{eqn. 5})$$

Higher S indicates greater sensitivity of the strain at time t to the variation in developmental timing. While this measure of sensitivity is exceedingly limited, it highlights a very simple relationship between the form of viscoelasticity and the sensitivity of the system to perturbations in developmental timing: S is equal to the parameter β in equation 4. In this viscoelastic model, the parameter β determines whether the material behaves more like a solid (β close to 0) or more like a fluid (β close to 1)

In our example, we would expect a morphogenetic process involving tissues that behave more like elastic-solids to be less sensitive to experimental variations in developmental timing (the timing of force generation in tissue A relative to the competent period of tissue B) than would processes involving tissues that behave more like viscous-liquids. Experimentally, such timing variations could be driven using thermal gradients (Black, 1989, Niemuth and Wolf, 1995, Lucchetta et al., 2008).

We might also consider how the viscoelastic behavior of the embryo could influence buckling behavior. If we consider the simple snap-through mechanism described above (Figure 2) it is clear that elastic and viscous materials would behave quite differently. For an elastic material, loads less than the critical load will not lead to snap-through; however loads higher than the critical load may not produce substantial deformations past the second stable position. On the other hand, a viscous material would not be stable in the initial position or the final position, since viscous materials – by definition – do not resist strain, but rather strain rate. The net result is that if we applied a short step of force for a period of time, the resulting tissue position would be much more strongly dependent on variation in both force magnitude and time in the viscous case (Figure 2C). So the stability and robustness of specific physical mechanisms of morphogenesis will likely depend strongly on the viscoelasticity of the tissue.

We should not leave the impression that viscous materials are inherently less robust to perturbations than are elastic materials. Systems in which a tissue closes an opening (e.g. dorsal closure in *Drosophila* or blastopore closure in *Xenopus*) may be cases in which a viscous material would more reliably reach a given end point than would an elastic material. In this case an elastic material would require a minimum amount of force to close the opening. Below that force, the opening would always remain, no matter how long the force is applied. However a viscous material would eventually close the opening with any given force, exerted in the right direction, if given enough time. So the process of closing an opening should be more robust to variation in the driving force if the material is viscous than if it is elastic.

These considerations imply that the particular features of the material model can influence the amount of variation in the resulting morphology. It is worth noting that many mechanical simulations of morphogenesis pick one of two extremes in material behavior: either purely

elastic ($\beta = 0$) or purely fluid ($\beta = 1$). In the former, temporal variation would have no effect; in the latter, temporal contributions to variation would be uniform over time. Real tissues typically show intermediate degrees of viscoelasticity. For example $0.15 \leq \beta \leq 0.45$ in the *Xenopus* gastrula epithelium (von Dassow et al., 2010), a range typical for cells (Fabry et al., 2001, Fabry et al., 2003, Lenormand et al., 2004, Lenormand and Fredberg, 2006, Zhou et al.).

Evidence from cell and protein gel studies indicate that the parameters of viscoelasticity can be tuned by the degree of actin cross-linking, the type of cross-linker, and myosin activity to be not just more or less deformable, but also more or less fluid-like (Tseng et al., 2005, Kasza et al., 2009, Koenderink et al., 2009, Sunyer et al., 2009b). By the same reasoning as discussed above for coupling between force generation and resistance to deformation, this suggests that the form of viscoelasticity, and the covariation among the mechanical parameters of the cell or embryo, are under genetic control. Therefore, we hypothesize that selection could adjust the form of viscoelasticity to reduce the sensitivity of morphogenesis to perturbations.

(4a) Mechanical signaling

No discussion of the role of mechanics in morphogenetic variation would be complete without discussing how mechanical stimuli could *induce* morphogenetic movements. Recent demonstrations of the importance of mechanical factors in altering cell movement, (Toyoizumi and Takeuchi, 1995, Pelham and Wang, 1997, Lo et al., 2000), force generation (Choquet et al., 1997, Lo et al., 2000, Paszek et al., 2005), and gene expression (Wang et al., 2003, Engler et al., 2006) has invigorated interest in old hypotheses that mechanical signaling could coordinate morphogenesis in the embryo (Toyoizumi and Takeuchi, 1995, Beloussov et al., 2006, Nerurkar et al., 2006, Hufnagel et al., 2007, Taber, 2008, Fernandez-Gonzalez et al., 2009, Pouille et al., 2009, Solon et al., 2009, Shindo et al., 2010). By mechanical signaling we mean that mechanical signals induce changes in biochemical states, whether that change in state results from a mechanotransductive pathway or a change in the activity or distribution of a single protein.

While the hypothesis that mechanical signaling coordinates embryonic morphogenesis is very enticing, it is not yet clear how common this phenomenon is. Most experiments to test this hypothesis have used cutting or laser ablation to alter the distribution of forces within the embryo (Beloussov et al., 2006, Fernandez-Gonzalez et al., 2009, Solon et al., 2009). Unfortunately, most studies have not adequately considered alternative explanations for the observed effects. Wounding could clearly result in chemical or electrical signals that lead to alterations in the cytoskeleton and tissue contractility (Clark et al., 2009, Joshi et al., 2010). Multiple recent studies have begun to investigate this hypothesis in *Drosophila* (Farge, 2003, Supatto et al., 2005, Fernandez-Gonzalez et al., 2009, Solon et al., 2009). One group studying elongation of the *Drosophila* germ band found that load applied using a micropipette resulted in cytoskeletal reinforcement after 70 seconds, whereas release of stress by laser ablation resulted in cytoskeletal reduction during the first 50 seconds (Fernandez-Gonzalez et al., 2009). However, because the time scale of the laser ablation test was short relative to the micropipette suction test, it remains possible that the responses to the two opposed perturbations were identical, rather than opposed.

Despite these limitations there is increasing evidence that mechanical signaling coordinates morphogenesis in some cases, in particular during the initiation of *Drosophila* ventral furrow and stomodeum formation (Farge, 2003, Supatto et al., 2005, Desprat et al., 2008, Pouille et al., 2009), as well as during the early morphogenesis of the chick heart (Nerurkar et al., 2006). One group investigating the role of force during invagination of the *Drosophila* ventral furrow found that mechanical force appears to trigger invagination (Pouille et al.,

2009). In other studies, they found clear evidence that compression of the anterior of the embryo by elongation of the ventral side affects gene expression in the presumptive mouth (Farge, 2003, Supatto et al., 2005, Desprat et al., 2008)

In one form of this hypothesis, mechanical signaling is assumed to occur in a specific, spatially localized group of cells that are capable of responding to a mechanical stimulus at a specific time period. For example, Odell et al (Odell et al., 1981) hypothesized that cells of the ventral furrow in *Drosophila* might contract in response to stretch, but that the surrounding cells would not. In that case a small initial contraction would propagate into a coordinated contraction driving ventral furrow invagination. Support for this hypothesis comes from experiments in which invagination is blocked in mutants that lack an initial random phase of contraction, but can be rescued by mechanical indentation (Pouille et al., 2009).

A more radical hypothesis proposes that mechanical signaling occurs among all parts of the embryo, and that each part changes its mechanical behavior (e.g. force generation or movement) in response to the mechanical signals. In this hypothesis, mechanical stimuli coordinate morphogenesis globally, throughout the whole embryo. This hypothesis has been extensively developed by Beloussov and co-workers for the frog embryo, in the form of "hyper-restoration" (Beloussov and Grabovsky, 2006, Beloussov et al., 2006), and has been extended by Taber (Taber, 2008, Taber, 2009). Beloussov and colleagues argue that changes in form of the *Xenopus* which result from cuts to the embryo are explainable by this model, and has found that deforming explants results in changes in explant morphology consistent with the model (Kornikova et al., 2010).

While we find such global mechanical signaling models intriguing, we are also puzzled by the fact that explants from frog embryos often show the same cellular behaviors as they would in the embryo (Shih and Keller, 1992a, Shih and Keller, 1992b, Keller et al., 2000, Davidson et al., 2004). In addition, removing the stiff vitelline membrane, which surrounds and the frog embryo, allows the embryo to slump greatly under its own weight relative to embryos left within their vitelline membrane (Fig. 3). Given the hypothesis of global mechanical signaling, we would expect such deformations to have a substantial effect on morphogenesis, yet embryos can develop quite well without their vitelline membrane (Fig. 3).

However there is substantial evidence that mechanical signaling could coordinate morphogenesis over long distances in at least one system. In *Drosophila* extension of ventral tissues (the germ band) around the posterior end of the embryo compresses the anterior of the embryo (Supatto et al., 2005). This compression appears to control gene expression in the presumptive mouth tissues in the anterior of the embryo (Farge, 2003, Supatto et al., 2005, Desprat et al., 2008). In addition, gentle compression of the embryo was sufficient to upregulate expression of the Twist protein in the whole embryo, suggesting that the whole embryo is sensitive to mechanical stimuli (Farge, 2003). Because these studies used a combination of multiple approaches to alter tissue deformation and loading, not just cutting, they provide a compelling case that mechanical signaling has a role in long-range interactions among parts of the *Drosophila* embryo.

Further work clearly needs to be done to investigate these hypotheses, however it would be of interest to investigate how mechanical signaling would affect phenotypic variation. Of particular value would be studies on how these models behave in the presence of variation in embryo size or tissue movements (Ewald et al., 2004), and noise from short time-scale or small spatial-scale mechanical variation within the embryo, for example due to cell divisions or contractions (Wallingford et al., 2001, von Dassow and Davidson, 2009). Quantitative

studies of morphogenetic variation in the embryo will be required for understanding the role of mechanical signaling and other processes in generating or suppressing noise in morphogenetic movements.

(4b) Function-dependent development and developmental adaptation

Although the role of mechanical signals in coordinating morphogenesis in the early embryo is still unclear, the role of mechanics in coordinating tissue growth, remodeling and shape change in many tissues from later developmental stages or adult organisms is well documented. Many biological systems, ranging in scale from the cytoskeleton to animal colonies, show feedback between the way they function in their environment and the way they develop (von Dassow, 2006).

An excellent example is the vertebrate circulatory system in which the structure of the circulatory system controls patterns of blood flow, but the flow influences the size of the blood vessels (Kamiya and Togawa, 1980, Langille, 1995). Biological fluid transport systems are used for a very wide range of functions and exhibit very different forms of organization (Labarbera, 1990, Vogel, 1994). For example some are used for oxygen transport while others are used for suspension feeding; some consist of networks of pipes with a central pump, and others are decentralized with multiple distributed pumps, and interconnected spaces as conduits for fluid flow. Despite this diversity, they share similar organizational principles based on their shared dependence on the physics of fluid flow (Labarbera, 1990, Vogel, 1994). Perhaps because of this, very similar feedback processes, in which increasing flow through the system leads to increased size or number of the conduits for fluid flow, appear to occur in very disparate biological fluid transport systems (von Dassow, 2006). Some of these systems are found in colonial animals (hydroids: (Dudgeon and Buss, 1996, Buss, 2001); bryozoans: (von Dassow, 2006)) and some are found intracellularly the plasmodium of slime molds (Nakagaki et al., 2000, Tero et al., 2008). Similar processes may also be involved in the development of the vertebrate heart (Hove et al., 2003).

Feedback loops between structure and function in systems used for mechanical support are also strikingly similar across a wide range of biological scales. The organization of collagen by fibroblast traction (Harris et al., 1981, Stopak and Harris, 1982) is highly reminiscent of the organization of actin filaments by myosin contractility (Rossier et al., 2010), and results in very similar appearing structures at cellular and higher scales. There are also striking similarities between the stress-dependence of bone growth and remodeling and the stress-dependence of wood growth (Mattheck, 1994, Moulia et al., 2006).

It is tempting to speculate that these feedback loops, which couple physiological function with growth and remodeling, edit the rough draft produced by embryogenesis and organogenesis to form a functional organism. It is noteworthy that the performance of fluid transport systems depends tightly on the dimensions of the conduits, with resistance scaling as vessel radius to the 4th power, so that small changes in morphology will produce large effects on performance (Zamir, 1977). The performance of supporting beams is similarly tightly dependent on the dimensions of the beam (Wainwright et al., 1976). However, despite some studies looking at the effects of these feedback processes on morphological variability, especially in mammal skulls (Zelditch et al., 2004, Willmore et al 2006, Zelditch et al., 2006, Zelditch et al., 2009), it is still unclear in general how they might influence variability in performance, which is what matters to the organism. We speculate that they tend to reduce variation in performance, while increasing variation in morphology (by tying it tightly to variation in the environment (West-Eberhard, 2003)), but this is neither certain nor obvious given the complexity of the systems involved. However, given the similarity of these feedback processes between such disparate systems as slime mold plasmodia and

human blood vessels, or between trees and bones, we expect that broad comparative studies would provide a great deal of insight into their effects on system performance, and into the dynamics of the feedback process.

(5) Structure from chaos

Since experiments with regeneration in hydra in the eighteenth century, biologists have recognized that in some organisms development can proceed to the same end point from vastly different starting points. Although the most popular model systems – *Drosophila*, *C. elegans*, zebra fish and mouse – show very limited capacities in this regard, many organisms are remarkable for their ability to produce similar structures by divergent pathways. Classic examples include regeneration in hydra, planaria and newts, as mentioned above, as well as the famous ability of sponges and hydra to reform new functional organisms from disassociated cells (Harris, 1987). Many animals from vastly different taxa distributed across the metazoan tree are capable of forming bodies – in some cases including very complex organ systems – by at least two very different modes of development: from eggs or by clonal budding. Examples include numerous cnidarians, bryozoans, urochordates, hemichordates, and echinoderm larvae, among others (Ruppert and Barnes, 1994).

Perhaps the most striking example occurs in botryllid ascidians – members of the same phylum as vertebrates. Some of these colonial ascidians can form new adult bodies (zooids) – complete with nervous system, gut, and heart, among other complex structures – by at least three completely different modes (Oka and Watanabe, 1957, Lauzon et al., 2002, Rinkevich et al., 2007, Voskoboynik et al., 2007, Brown et al., 2009): a highly stereotyped developmental pathway starting from an egg, which produces a larva, and then metamorphoses into a feeding zooid attached to a substratum; budding of new zooids off of existing zooids at specific regions of an epithelium; or aggregation of circulating blood cells.

These systems pose a fundamental challenge to our understanding of development in general, and the physics of morphogenesis in particular. Numerous processes at the level of signaling, patterning, stem cell regulation, and specification of cell fates (e.g. (Rinkevich et al., 2007, Voskoboynik et al., 2007, Brown et al., 2009) are likely to be extremely important in many if not all of these instances in which development can produce the same morphology from vastly different starting points. Certainly re-specification of cell fates has been shown to be very important in the regulative capacity of echinoderm embryos to various experimental perturbations (e.g. (McClay and Logan, 1996). However, these biological systems, in which the geometry of development can vary tremendously in the same species, but produce the same result, are puzzling from a mechanics standpoint as well. Even fairly robust mechanical models typically require some similarity in the boundary conditions and tissue geometry. Therefore, understanding the physical processes involved in these fascinating biological systems would aid in developing strategies for producing artificial tissues and organs.

Studies on the sorting of disassociated cells inspired Steinberg and colleagues (Steinberg, 1963, Foty et al., 1996, Foty and Steinberg, 2005) to propose the differential adhesion hypothesis (DAH). Harris suggested a number of alternatives, including some simple modifications to the DAH that would make it more physically plausible (Harris, 1976). These studies and more recent work extending these original models (Krieg et al., 2008, Manning et al., 2010) suggest that there could be several different physical mechanisms that can bring a system to the same configuration despite starting from a wide variety of initial configurations. Many of these mechanisms involve surface tension like forces (due to a variety of physical mechanisms) acting on a more or less fluid-like material. However, chemotaxis could also produce similar patterns by different mechanisms (Trinkaus, 1984);

and other physical mechanisms not discussed above may also produce similar phenomena. These studies have already inspired strategies for tissue engineering (Jakab et al., 2006).

Studies of echinoderm larval cloning – in which larvae bud off new larvae at different developmental stages (Eaves and Palmer, 2003, Vaughn, 2009, McDonald and Vaughn, 2010) – and ascidian budding are likely to be of particular value in illuminating the question of how organisms can develop to the same morphology along multiple different pathways. Echinoderms have advantages in that much is already known about their early development (including their regulative capacity: (Ettensohn and Malinda, 1993, McClay and Logan, 1996)), they are amenable to mechanical studies (Mitchison and Swann, 1954b, Gustafson and Wolpert, 1963, Ettensohn, 1984, Hardin and Cheng, 1986, Davidson et al., 1999, Takata and Kominami, 2001a, Takata and Kominami, 2001b), and they are more closely related to chordates than the most popular invertebrate model systems, *Drosophila* and *Caenorhabditis*, are (Halanych, 2004). Ascidians have advantages due to the great variety of ways they can undergo development to form an adult body, and because they are very close relatives of vertebrates (Halanych, 2004). Temperature gradient studies as cited above may also prove useful in more traditional model systems.

Perspective

Understanding how morphogenesis responds to natural variation, and in turn how the physics of morphogenesis affects phenotypic variation, will be valuable from both scientific and practical standpoints. On the one hand this will aid in understanding the evolution of development by clarifying how genetic variation maps onto phenotypic variation (a prerequisite for natural selection). It will also provide insight into the reasons why the mechanisms of morphogenesis are what they are, because these mechanisms have evolved to function in the context of environmental variation. On the other hand it will aid in identifying mechanisms that generate or prevent developmental defects. It may identify combinations of circumstances that would increase the likelihood of defects, even when those factors are of limited effect on their own. For example, a small change in the time dependence of viscoelasticity (e.g. a small increase in β in equation 4) might increase the sensitivity of morphogenesis to perturbations in developmental timing driven - perhaps - by changes in temperature or oxygen availability. Mutations that affect the expression levels of actin cross-linkers could alter the coupling of force generation to viscoelastic resistance, and thereby change the sensitivity of the morphogenesis to other mutations or toxins which affect force generation or cell viscoelasticity. In our work, we have seen remarkably little effect on morphogenesis of some drugs that greatly change tissue mechanics. However, it seems possible that these drugs might push embryos on the softer end of the range of natural variation in deformability, or at the extreme ranges of size, over a threshold to having developmental defects.

We have suggested a few of the many possible factors that might impinge upon the physics of morphogenesis in natural populations, and a few of the features of morphogenesis that might contribute to mapping this variation onto the resulting phenotypic variation, and ultimately onto variation in performance. We suggest that developmental biologists should begin to (1) identify those perturbations that both impinge on the mechanics of morphogenesis and which organisms are likely to experience in nature; (2) determine how the physics of morphogenesis and simultaneous gene regulatory processes map these perturbations onto variation in morphology, and (3) consider how that variation in morphology affects subsequent development and – ultimately – alters how the organism functions (Figure 4). Doing these three things should aid in mapping genetic and environmental variation onto phenotypic variation.

Birth defects occur with relatively low frequency so identifying even major single factors that contribute to their frequency can be quite challenging (Chambers et al., 1998, Acs et al., 2005, Moretti et al., 2005). Given that they are likely to be produced by multiple, complex, interacting factors (Acs et al., 2005, Copp and Greene, 2010), identifying combinations of factors that together increase risk is a great challenge. We expect that developing a combination of novel biological model systems and theoretical tools that can identify combinations of variables that could combine synergistically to cause developmental problems would be valuable in solving this challenge. We expect this to be equally true for studying the effect of pollutants on other species.

To understand the physics of development, we must consider not just the scales from molecules to tissues, but also higher scales, including the way the organism functions, the organism's environment, variation with the population, and evolution. In addition, broadening the focus from highly inbred laboratory animals to other organisms would provide new perspectives on old questions as well as inspiring new questions.

Box 1: A note on terminology

Many common English words are used with very narrow senses in engineering jargon. Furthermore, there can be several different but closely related properties that are appropriate for different types of situations (e.g. the creep compliance versus relaxation modulus of viscoelastic materials). This can make discussion of the simple and general relationships that we are primarily concerned with in this review unnecessarily confusing.

Therefore we take “resistance to deformation” to mean the most generic proportionality between a deformation of some kind occurring at one time and a force (or stress), applied at an earlier time. For a linear, isotropic, purely elastic material, the resistance to deformation could be measured as the Young’s modulus, shear modulus, or bulk modulus depending on the type of applied stress. For a structure such as a beam, it could be a structural property such as flexural stiffness. Strictly speaking a fluid resists the rate of deformation rather than the magnitude of deformation (as discussed under “(3) the form of viscoelasticity”). However, we can reasonably describe a fluid as having a resistance to deformation as long as we restrict ourselves to the amount of deformation at a specified finite time after the time of force application. Therefore, for a fluid we can take the viscosity as a measure of the resistance to deformation. For viscoelastic materials, resistance to deformation would be the time dependent relaxation modulus or the inverse of the creep compliance (at some chosen time after application of load), depending on which form is more appropriate for the situation.

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Figure 1. Size variation in *Xenopus laevis*

A) Eggs laid by different females can differ in their diameter. Egg size variation is often overlooked unless two clutches are directly compared side-by-side. A clutch of normal sized eggs (Diameter: 1.42 ± 0.03 mm, mean $\pm$ SD) is shown in the left half of the panel, and a clutch of large eggs (1.77 ± 0.02 mm) is shown in the right half panel. B) Early cleavage stages show that the large- and normal-sized eggs can undergo synchronous division cycles. C) Rounds of successful cell division produced late blastula stage embryos with indistinguishable patterns of animal cap ectoderm. D) Subsequent development through gastrulation showed differences in the formation of the bottle cells and closure of the blastopore (large embryo: lower; normal sized: upper). However, once these gastrulation movements advanced the process of neurulation appeared unchanged. E) Large and normal sized embryos appeared to neurulate at the same time. F) As development progressed tadpoles derived from large eggs appeared more similar in length but partition more yolk cells into ventral tissues. G) Size differences in the early "round-embryo" blastula stages were reduced once the body plan was shaped and the embryo elongated. Eggs and embryos in B, C, and E are mixed to highlight difference in size.

Figure 2. A mechanical analogy for robust morphogenesis due to limit point buckling in an elastic tissue

Two hinged beams comprised of either elastic (A) or viscous (B) material will show quite different behavior (C) when loaded by a force (F) along the gray arrow. If one looks at the position of the central point (lower part of C) after application of a square pulse of force (upper part of C), one gets very different behaviors for the two models. For any force below a critical force, the elastic model (solid black line) will be in the original configuration; above that critical force, the elastic model will snap into a new configuration. The viscous model (dashed lines) can take on any position, depending on both force magnitude and duration (the separate lines) of the pulse.

Figure 3. Normal development can occur despite deformation of the *X. laevis* embryo

A pair of embryos were filmed in a mirror (A, B, and C) to image development from the side. The same pair of embryos was imaged from above in D. The embryo on the left had the vitelline membrane removed at Stage 8 and slumped under its own weight, but completed gastrulation and neurulation without noticeable defects; the embryo on the right had an intact vitelline membrane and remains round through gastrulation.

Figure 4. Scheme for integrating organism-scale and molecular-scale processes in developmental biomechanics

Processes at the molecular, cellular and tissue scale map stochastic, genetic, and environmental variation onto variation in mechanical variables (including developmental timing and geometry) that drive morphogenesis; the physics of morphogenesis maps the resulting variation into variation in morphology; this in turn is mapped onto variation in performance. The graphs illustrate hypothetical covariances among a few of the variables that might be relevant for *Xenopus*. Arrows indicate possible instances of feedback (e.g. mechanotransduction, changes in inductive events or geometry at earlier developmental stages, or developmental adaptation). Naturally, not all combinations of factors or connections are shown.